

On page 2 of the October 2002 Office Action, the Examiner points out certain deficiencies in Applicants' manner of making the specification amendments filed in the July 2002 Response. In particular, the Examiner notes that Applicants directed amendments to three single lines in the specification, and that on or after March 1, 2001, changes to paragraphs may only be made by complete replacement (37 CFR 1.111 and 1.121).

Applicants are hereby amending the specification in accordance with 37 CFR 1.111 and 1.121. These amendments are to correct certain informalities in response to objections to the specification made by the Examiner in the January 2, 2002 Office Action, and other typographical errors identified by Applicants. Attached hereto is a marked-up version of the changes made to the specification by the current amendment (the attachment entitled "Version with markings to show changes made."). No new matter is being added.

This reply is responsive to every ground of objection in the October 2002 Office Action. This reply and the July 2002 Response are together responsive to every ground of objection and rejection in the Office Action dated January 2, 2002. Favorable consideration of the new Claims 11-20, filed in the July 2002 Response, is respectfully requested. Should any matter require resolution before allowance of these claims, the Examiner is asked to contact the undersigned attorney at the telephone number listed below.

Respectfully submitted,



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VERSION WITH MARKINGS TO SHOW CHANGES MADE*

* deletions shown by strike-through and additions shown by double-underline

IN THE SPECIFICATION:

Page 3, first full paragraph (lines 1 - 12):

These chelates have been described as having chemical stability, long-lived fluorescence (greater than 0.1 ms lifetime) after bioconjugation and significant energy-transfer in specific bioaffinity assays ~~US 5,162,508~~ U.S. 5,162,508, issued to Lehn, et al. on November 10, 1992 discloses bipyridine cryptates. Polycarboxylate chelators with TEKES type photosensitizers (EP 0203047 A1) and terpyridine type photosensitizers (EP 0649020 A1) are known. International Publication No. WO 96/00901 of Selvin et al., having an International Publication Date of January 11, 1996, discloses diethylenetriaminepentaacetic acid (DTPA) chelates which used carbostyryl as sensitizer. Bailey, et al., Analyst, **109**, (1984) 1449; Ando, et al. Biochim. Biophys. Acta, **1102**, (1992) 186; and Heyduk et al., Anal. Biochemistry, **248**, (1997) 216 also describe DTPA lanthanide chelates which contain different sensitizers. Additional DTPA chelates with other sensitizers and other tracer metals are known for diagnostic or imaging use (e.g., EP 0450742 A1).

Page 11, Example 1 (lines 10 - 25) replacement:

Example 1

Preparation of 3AAP-DTPA (1) and 3AAP-DTPA-4APEA (5)

To a solution of ~~DTPAA~~ DTPA (143 mg, 0.4 mmol) in 10 mL dry DMSO and 2 mL dry triethylamine was added a solution of 3-aminoacetophenone (3AAP, 54 mg, 0.4 mmol) in 5 mL DMSO. The mixture was stirred at room temperature for 0.5 h and then treated with a solution of 4-aminophenethylamine (4APEA, 53 mg, 0.4 mmol) in 5 mL DMSO. The mixture was allowed to stir at room temperature for an additional 3 h and then evaporated to dryness. The oily residue was chromatographed on reversed-phase C18 hplc (using a step gradient of 0 to 60% acetonitrile in 0.1% TFA buffer) to give, after lyophilization, **1** as a cream colored solid and **5** as a pale yellow solid. Compound **1** was obtained in 59 mg yield. ¹H-NMR (CD₃OD) : δ 2.60 (3H, s), 3.1-3.5 (10H, m), 3.6 (2H, s), 3.65 (2H, s), 3.71 (2H, s), 4.42 (2H, s), 7.42 (1H, dd), 7.75 (1H, dd), 7.83 (1H, dd), 8.31 (1H, d); MS: m/z 511 (M-H), Compound **5** was obtained in 16 mg

yield. $^1\text{H-NMR}$ (CD_3OD): δ 2.62 (3H, s), 2.73 (2H, t), 3.21 (2H, t), 3.3-3.55 (12H, m), 3.65 (2H, s), 3.74 (2H, s), 4.35 (2H, s), 7.13 (4H, s), 7.41 (1H, dd), 7.75 (1H, dd), 7.83 (1H, dd), 8.32 (1H, d); MS: m/z 682 ($\text{M} + 3\text{NH}_4$), 683 ($\text{MH} + 3\text{NH}_4$).

Page 11 bridging page 12, Example 2 (page 11, line 27 to page 12, line 4) replacement:

Example 2

Preparation of 4AAP-DTPA-APEA-ITC (6).

To a solution of 4AAP-DTPA-APEA (**3**, 12 mg, 0.019 mmol) in 10 mL of 0.5 N HCl was added 4 mL of thiophosgene (85% in CCl_4). The two phase reaction was allowed to stirred vigorously for 1 h. The mixture was worked up by separating the layers in a separatory funnel and the ~~aqueous~~ aqueous solution was washed by additional methylene chloride and then chromatographed on a small reversed-phase C18 column to give the thioisocyanate product (**6**), an off-white solid in 10 mg yield after lyophilization. $^1\text{H-NMR}$ (CD_3OD): δ 2.60 (3H, s), 2.72 (2H, t), 3.20 (2H, t), 3.3-3.5 (12H, m), 3.65 (2H, s), 3.74 (2H, s), 4.34 (2H, s), 7.12 (4H, s), 7.41 (1H, ss), 7.74 (1H, dd), 7.84 (1H, dd), 8.20 (1H, d); MS: m/z 724 ($\text{M} + 3\text{NH}_4$), 725 ($\text{MH} + 3\text{NH}_4$); IR: 2108 cm^{-1} ($\text{S}=\text{C}=\text{N}$ stretch).

Page 12, Example 3 (lines 6 - 20) replacement:

Example 3

Preparation of 4ABP-DTPA (4**) and 4ABP-DTPA-4APEA (**12**)**

To a solution of ~~DTPAA~~ DTPA (179 mg, 0.5 mmol) in 5 mL of dry DMSO and 3 mL of dry triethylamine was added a solution of 4-aminobenzophenone (4ABP, 99 mg, 0.5 mmol) in 5 mL DMSO. The mixture was stirred for 0.5 h and treated with a solution of 4-aminophenethylamine (4APEA, 68 mg, 0.05 mmol) in 5 mL DMSO. After an additional 3 h stirring at room temperature, the mixture was evaporated to dryness. The oily residue was chromatographed on reversed-phase C18 hplc (using a step gradient of 0-60% acetonitrile in 0.1% TFA buffer) to give **4** as a cream colored solid and **12** as a pale yellow solid. Compound **4** was obtained in 57 mg yield. $^1\text{H-NMR}$ (CD_3OD): δ 3.2-3.5 (10H, m), 3.60 (2H, s), 3.63 (2H, s), 3.74 (2H, s), 4.43 (2H, s), 7.53 (2H, m), 7.62 (1H, dd), 7.76 (2H, m), 7.8 (4H, s); MS: m/z 573 ($\text{M} + \text{H}$). Compound **12** was obtained in 47 mg yield. $^1\text{H-NMR}$ (CD_3OD): δ 2.73 (2H, t), 3.25

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(2H, t), 3.3-3.5 (12H, m), 3.67 (2H, s), 3.73 (2H, s), 4.3 (2H, s), 7.23 (4H, s), 7.55 (2H, m), 7.64 (1H, dd), 7.8 (2H, m), 7.83 (4H, m); MS: m/z 691 (M+H).